HYPOCHOLESTEROLEMIC AND ATHEROGENIC EFFECT OF LEGUMES VERSUS ANIMAL PROTEIN: Review of animal and human studies

Susilowati Herman*

ABSTRAK

Kedelai mempunyai peranan penting dalam budaya bangsa-bangsa di Asia, baik sebagai makanan maupun obat-obatan. Di negara-negara Barat, kedelai dikenal karena kandungan protein yang cukup tinggi. Dari sudut pandang gizi, protein kedelai mempunyai kelebihan dibanding dengan protein hewani, selain kandungan asam lemak jenuh yang lebih rendah dan tidak mengandung kolesterol. Di samping itu kedelai juga mengandung isoflavon *yakni,* genistein, daidzein, *dan* glycetein. Genistein *mempunyai potensi antiaterogenik. Dari beberapa studi yang menggunakan protein kedelai untuk menggantikan seluruh atau sebagian protein dalam makanan subyek penelitian, memberikan hasil yang belum konsisten. Studi pada berbagai hewan percobaan, maupun pada subyek manusia memberikan hasil yang belum konsisten. Tulisan ini menelaah perkembangan penelitian tentang efek hipokolesterolemik dan aterogenik protein kedelai dan kacang-kacangan lainnya pada hewan percobaan dan pada manusia. Efek hipokolesterolemik leguminosa tidak tinggi dan kedelai mempunyai efek paling tinggi dibandingkan dengan leguminosa lain. Mekanisme hipokolesterolemi setiap komponen atau zat dalam makanan berbeda dan tampaknya terdapat pengaruh satu dengan yang lain. Kecenderungan studi yang akan datang yaitu mempelajari pengaruh diet pada kolesterol darah, dan bukan pengaruh masing-masing zat gizi. Dianjurkan bagi orang sehat untuk mengkonsumsi makanan yang bervariasi, seimbang, dan moderat.*

Kata kunci: kolesterol, aterosklerosis, protein hewan, leguminosa.

INTRODUCTION

Legumes, especially soybeans have played an integral part in Asian culture, both as a food and as a medicine, for many centuries. In China the word for soybean is *ta-tou*, which means "greater bean" [1]. This is not surprising given the importance soybeans have played in Asian culture, both as a food and as a medicine. In the West, soybeans are still best known for their protein content.

From the nutritional perspective, soy protein may hold advantages over animal proteins above and beyond the fact that soybeans are low in saturated fat, and of course, cholesterol-free. The nutritional quality of soybeans has long been understood by Asian cultures, where non-fermented and fermented soy are a major source of protein. Quality of soy protein has been documented by numerous animal studies and supported by amino acids composition determinations [2,3]. Of the many soy-based foods available, soy sauce and *tofu* are the major ones to have reached the supermarket. Soybeans contain two major isoflavones, genistein and daidzein, and a minor one, glycetein. In the seed, the

* Pusat Penelitian dan Pengembangan Gizi, Depkes RI.

isoflavones are present primarily as β-glucosides and a portion of the glucosides also are substituted on the C-6 hydroxyl of the glucose by a malonyl group, especially in the hypocotyls [4].

A dietary factor known to influence plasma lipids that has received little public attention is protein. It is stated frequently that dietary animal proteins produce higher serum cholesterol concentrations in rats than do vegetable proteins. However, essentially almost all studies on the effects dietary proteins on serum cholesterol in rats have focused on the comparison of soy protein and casein [5]. Studies in which soy protein has been substituted for all or part of dietary protein have shown mixed results [6]. Studies in different animal models and in man have provided inconsistent findings. Everything in the diet can affect cholesterolemia and experimental atherosclerosis. Dietary cholesterol has relatively little effect on blood cholesterol. Saturated fat is a major factor in cholesterolemia. In rabbits, triglyceride structure affect atherosclerosis but nor blood cholesterol. Protein effects is secondary to those of fat [7]. Here, we review some of the more recent studies on the hypocholesterolemic and atherogenic effect of soy-protein using animal model as well as human subjects.

STUDIES IN ANIMALS MODEL

The influence of dietary protein on experimental atherosclerosis was the basis of Ignatowski's early studies, but the intensity with which this topic was pursued in the early years of this century is not common knowledge. The early work was carried out to determine the effects of protein on renal metabolism and hypertension as well as on atherosclerosis. Ignatowski fed rabbit's meat, egg yolk, or milk and found that this diet resulted in anemia, renal pathological changes and atherosclerosis. He postulated that some toxics factors in animal protein or acidic products resulting from animal protein metabolism were the causes underlying the observed aortic atherosclerosis. Ignatoski's finding stimulated research designed to test whether his results were due to the protein or fat diet [8]. A lipid component of egg yolk was the atherogenic factor and set about to compare the effect of cholesterol and lecithin on atherosclerosis in rabbits. He concluded that cholesterol was the ahterogenic constituent in the egg yolk.

The rabbit has been the most popular and widely used animal models in the atherosclerosis research [9]. In most studies soybean protein has been compared with the animal protein, especially casein. Using cholesterol free, semi purified diets with the protein source as the only variable, soybean protein induce no hypercholesterolemia and atherosclerosis, whereas casein does [10]. Likewise, on diets containing other vegetables proteins such concentrations of serum cholesterol, whereas with whole egg protein, beef protein concentrate and pork protein concentrate elevated serum cholesterol are seen. Carrol and Hamilton studied cholesterolemic effects of a large number of proteins of plant or animal origin fed to rabbits for 30 days at a dietary level of 30%. Cholesterol levels in rabbits fed animal proteins ranged from 260 mg/dl (egg yolk) to 100 mg/dl (egg white). Among the rabbits fed plant proteins, cholesterol levels ranged from 30 mg/dl (fava beans) to 80 mg/dl (wheat gluten) [11]. The cholesterol lowering properties of soybean protein have also been demonstrated in various other animal models such as the rat, hamster, pig, and monkey [12].

Susilowati Herman et al. [13] reported that diets containing isolate vegetable protein such as soybean, mug bean, green beans could maintain serum cholesterol level when compared with animal protein such as casein, isolate fish protein, isolate beef protein, and isolate egg protein. Comparing the beans and their isolates, the isolate proteins were more hypercholesterolemic compared with the beans. Similar results were seen when comparing animal proteins with their isolates (Figure 1). Among animal proteins, fish protein and its isolate were more hypercholesterolemic than of egg, beef and casein. The changes of serum LDL and HDL cholesterol concentration of rabbits fed protein isolate shows that the highest changes of serum cholesterol concentration were found among fish protein isolate Figure 2). The highest changes of serum HDL cholesterol concentration were observed amongst rabbits fed egg protein isolate ($p<0,05$). Histopatology examination, plaque areas and plaque thickness of aorta of the rabbits shows that isolate animal protein (mackerel fish, beef, egg, and casein) more atherogenic compared with isolate plant protein (soybean, green beans, and mungbean). Atherosclerosis formation was indicated by the foam cell (myointima cell containing lipid), fibrosis reaction, and sometimes it was followed by formation of fibrous cap, cholesterol cleft, from mild up to severe form. The relationship between the changes of total cholesterol levels, LDL cholesterol and the incidence of aterosclerosis, seems that the increment of total cholesterol (2 times higher compared with baseline) and LDL cholesterol (4 times higher compared with baseline) was observed on isolate green bean protein plus 1% methionine group was followed by the higher incidence of atherosclerosis significantly. Although amongst mungbean diet plus 1% methionine group the increment of total cholesterol (0,5 times higher compared with baseline) and LDL cholesterol (0,5 times higher compared with baseline) the higher incidence of aterosclerosis was not observed. This finding indicated that additional 1% methionine to the green bean and mungbean diet did not have any specific effect on the incidence of aterosclerosis. Isolate soybean diet and isolate soybean diet plus methionine 1% have the lowest atherogenicity compared with other isolate bean diets [13]. Other investigators provided evidence that LDL apolipoprotein B (apo B) synthesis is stimulated by a casein semi purified diet [14]. On the other study, Khosla, 1991 observed that the down-regulation of LDL receptors preceded the increase in plasma cholesterol [15].

Sugano et al, 1988 [16] reported that in rats the effect of casein versus soy protein on serum cholesterol levels was not influence by the composition of the background diet as to the use of fish oil or vegetable oil as fat source. However, other researchers reported that the hypocholesterolemic effect of soy protein was not evident when sardine oil was fed as a fat source [17]. Eklund and Sjoblon, 1985 reported that there was no significantly effect of dietary protein on plasma cholesterol of rats fed diets containing less than 0.25% cholesterol. For diets containing 0.5% and 1% cholesterol, however, a much more pronounced hypercholesterolemic response was observed with casein compared with soybean protein. The casein diet containing 2% cholesterol did not result in any further increase in the plasma cholesterol level. Perhaps this phenomenon is due to a limitation in the rate of intestinal absorption of cholesterol [18].

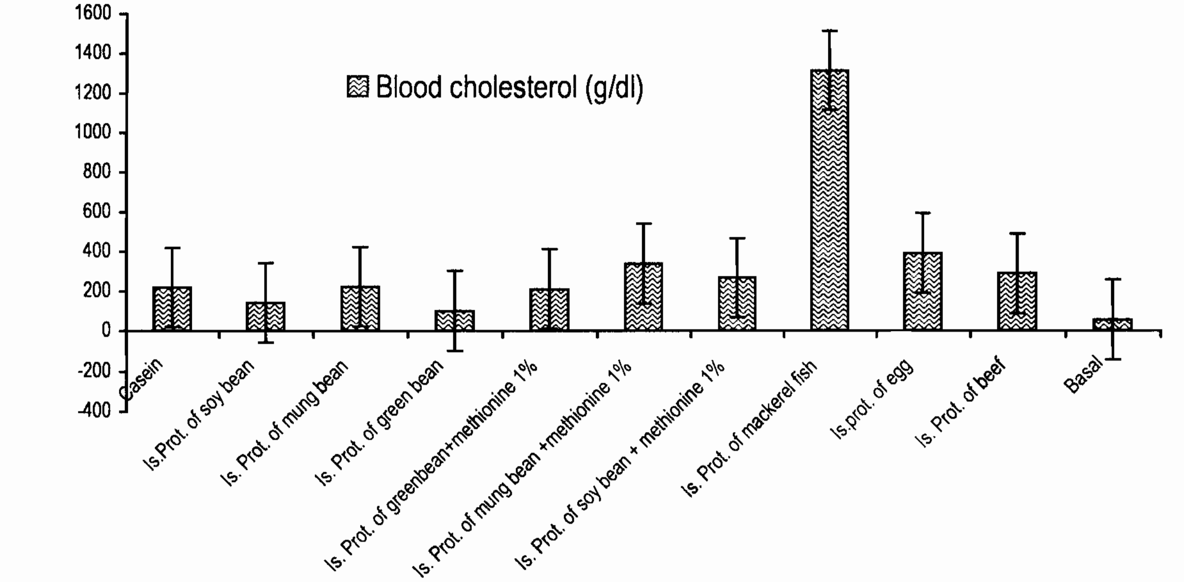

Figure 1. Cholesterol Changes of the Rabbits at the End Experiment Treatment[1]
Source: Susilowati, H et al. (2001)

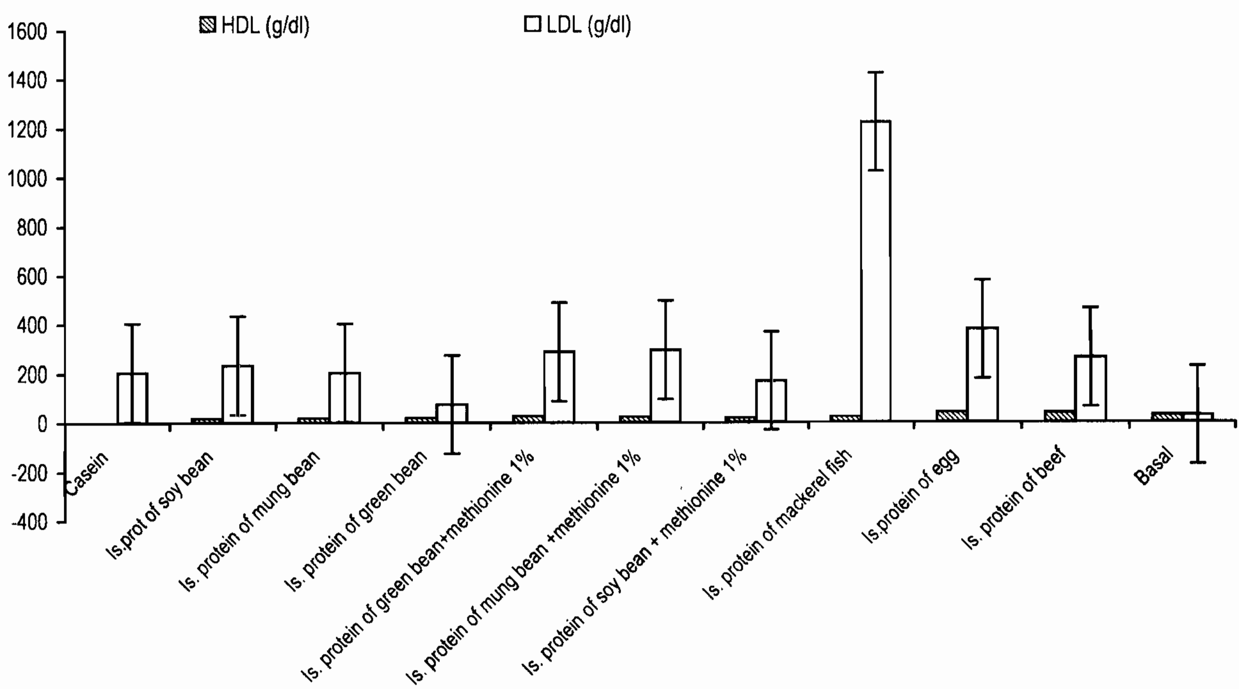

Figure 2. LDL and HDL Cholesterol Changes of the Rabbits at the End Experiment Treatment[1]
Source: Susilowati, H et al. (2001)

Soy proteins have a potential to modify atherosclerotic development through a number of different pathways. Soy proteins have been shown to reduce serum cholesterol concentrations in experimental animals fed a high-fat diet [13,19]. This reduction is thought to be due to the reduced absorption of steroids in the gastrointestinal tract, presumably by increasing binding to bile acids and excretion [20,21,22,23].

High-protein diets are more atherogenic than low-protein diets in squirrel monkeys. Freyberg fed rabbits diets containing 13.1, 33.0, or 37.8% of vegetables protein and found no atherosclerosis. The terminal serum cholesterol was 79 ± 9, 82 ± 11, and 119 ± 19 mg/dl in the three groups. Freyberg also found no difference in atherosclerosis in rabbits fed 25.0 or 37.5% soy protein [24]. Meeker and Kesten show that animal and vegetable protein (represented by casein and soy protein, respectively) possessed vastly different atherogenic potential (Table 1). They fed rabbits diets containing 38% protein with or without cholesterol for 6 months. Casein was more atherogenic in the absence of cholesterol than soy protein was in rabbits fed casein and 60 mg day of cholesterol was six times as severe as that seen in rabbits fed the soy plus cholesterol diets [25,26].

Casein was shown to be more cholesterolemic than soy protein when the two proteins were fed to rats at levels of 10, 20, 40, or 60% in the diets than also contained cholesterol and cholic acid. Hamilton and Carroll demonstrated that a wide variety of animal proteins were more cholesterolaemic for rabbits than vegetables proteins and this work supported the generalization that animal protein was the more cholesterolaemic protein [27]. The type of protein present in the diet may interact with other dietary components such as fiber. Rabbits fed semi-purified cholesterol free diet containing casein and cellulose were significantly more cholesterolemic and 20% more atherosclerotic than rabbits fed soy protein and cellulose. When the fiber was alfalfa, the effects of the two proteins were equivalent.

Table 1. Influence of Casein and Soy Protein on Atherosclerosis in Rabbits.

Experiment	Basal	Casein	Soy protein
Experiment 1:			
Survival	8/8	6/6	-
Cholesterol (mg/day)	-	-	-
Mean atherosclerosis	0.0	0.67	-
Experiment 2:			
Survival	9/10	8/8	-
Cholesterol (mg/day)	60	60	-
Mean atherosclerosis	0.89	2.00	-
Experiment 3:			
Survival	9/9	-	6/8
Cholesterol (mg/day)	60	-	60
Mean atherosclerosis	0.89	-	0.33
Experiment 4:			
Survival	6/6	-	6/6
Cholesterol (mg/day)	250	-	250
Mean atherosclerosis	1.50	-	0.67

Atherosclerosis is graded on a scale of severity 0-3.
Source: Meeker, D.R. and Kersten H.H. (1940) ; and Meeker, D.R. and Kersten H.H. (1941).

When compared with dietary soybean protein, casein produced hypercholesterolemia in rabbits but the effect of fish protein more pronounced [13,28]. After prolonged feeding of the proteins, a substantial part of the excess of cholesterol in serum was transported in the VLDL fraction in casein-fed rabbits, and in the LDL-fraction in animals fed fish protein. Casein caused increased concentrations of liver cholesterol, but fish protein did not. A similar effect was observed in rats. Casein depressed the fecal excretion of neutral steroids and bile acids in rabbits more markedly than did fish protein. It meant that casein and fish protein induce hypercholesterolemia through different mechanism.

Lapre et al, 1988 [5] reported that dietary protein had no effect on serum cholesterol concentration. Group mean liver cholesterol was increased and fecal excretion of bile acids was decreased by all animal proteins (casein, whey protein, fish protein, hemoglobin, plasma protein, ovalbumin, egg-yolk protein, beef protein, and chicken meat protein) when compared with soy protein. This study revealed that carefully balancing diets for components other than protein in the protein preparations prevents protein effects on serum cholesterol in rats but not on liver cholesterol and bile acid excretion. It has been shown that rabbits fed soy protein excrete more cholesterol and absorb less than those fed casein. Plasma cholesterol and lipoprotein turns over more rapidly in rabbits fed soy protein. These finding, which would be expected from observed differences in serum cholesterol levels and atherosclerosis, still do not offer clues as to which differences in protein composition underlie the experimental results. Some researchers suggested that the differences between casein and soy protein might be due to their lysine arginine ratios. The lysine/arginine ratio of casein is about 2, and that of soy protein is about 1. Experiments in which rabbits were fed casein, casein plus arginine (to give lysine/arginine ratio of 1), soy protein, and soy protein plus lysine (to raise the lysine arginine ratio of 2) have shown that addition of arginine to casein does affect cholesterolaemia but reduces average atherosclerosis by 24%. Conversely, addition of lysine to soy protein enhances cholesterolaemia by 53% and increases average atherosclerosis by 64%. The mechanism/s of the plasma cholesterol reduction induced by soy protein are, however, incompletely understood. The effect of mixtures of animal and vegetable protein was studies in an experiment in which beef protein and textured vegetable protein (TVP) were fed individually or together as part of a semi-purified cholesterol-free diet. Animal fed beef protein exhibited higher serum lipids and more severe atherosclerosis than those fed TVP. Rabbits fed beef protein: TVP (1:1) had serum cholesterol levels that were significantly higher than observed in rabbits fed TVP and significantly lower than those in rabbits fed beef protein. Average severity of ateromas (arch plus thoracic/2) graded on a visual scale of severity from 0 to 4 was: beef protein, 1.02; TVP, 0.50; and beef protein-TVP (1:1), 0.55.

Rabbits fed semi-purified diets containing casein or soy protein show other differences in cholesterol metabolism that could help to account for the fact that casein is hypercholesterolemic, where as soy is not [6,20,29]. There are two aspects of studies regarding the evaluation of soybean proteins in experimental hypercholesterolaemia namely: i) the

Tetranthera brawas Bl dari familia Lauraceae. Daunnya dapat digunakan sebagai obat penambah nafsu makan, membuat tenang, kolagogum. Di daerah Bogor daun trawas setelah diremas-remas kemudian dioleskan pada payudara ibu untuk menambah keluarnya air susu [7]. Kandungan kimia daun trawas terdiri dari minyak atsiri kuning muda (minyak trawas yang mengandung *nonyleen*, *Methyl keton* [CH_2=$CH(CH_2)_7$-$COCH_3$], *Methyl-n-nonyleen-Carbinol*) [8].

c. Rimpang Jahe (Zingiberis rhizoma)

Rimpang jahe berbau aromatik dan mempunyai rasa yang pedas [9]. Secara tradisional, jahe yang mentah diparut digunakan sebagai obat oles untuk mengobati pembengkakan atau rematik dan kadang-kadang juga untuk mengobati sakit kepala. Selain itu, juga digunakan sebagai karminatif, peluruh dahak (obat batuk), peluruh haid, peluruh keringat, pencegah mual, penambah nafsu makan dan penurun tekanan darah [5,7]. Rimpang jahe dengan minyak atsiri 2%--3% yang mengandung zingiberen, feladren, kamfer, limonen, borneol, sineol, sitral dan zingiberol. Juga terdapat minyak damar yang mengandung zingeron [9].

d. Kembang Pulu (*Catharmi flos*)

Kembang Pulu merupakan bunga majemuk *Catharmus tinctorius* L, mempunyai bau agak aromatik dan rasa agak pahit, biasanya digunakan sebagai laksatif, astringen. Kembang pulu mengandung zat warna merah cartamin (catharmic acid $C_{12}H_{22}O_{11}$), zat warna kuning (saflower $C_{22}H_{30}O_{15}$), lendir dan minyak lemak [10].

e. Jungrahab herbae (*Baeckeae folium*)

Jungrahab *herbae* merupakan bagian seluruh tumbuhan yang digunakan untuk pengobatan, mempunyai bau khas aromatik dan rasa agak pahit [6]. Digunakan sebagai diuretik, obat sakit perut, obat demam, demam nifas, abortivum, untuk mengusir serangga dengan baunya, bagian jamu (untuk membersihkan setelah haid), gigitan ular, malaria, cacing gelang, susah kencing, dan pengobatan pasca persalinan. Kandungan kimia dari Jungrahab adalah minyak atsiri (eugenol, kariofilen), damar, zat samak, glikosida pahit [6]

Hasil penelitian ini diharapkan dapat memberikan informasi ilmiah mengenai kemampuan suatu kandungan komponen jamu untuk berpartisi melalui membran biologik. Lebih jauh lagi untuk mengetahui koefisien partisi dan karakteristik kandungan komponen jamu nifas yang dapat berpartisi kedalam sistem *octanol* : air.

METODOLOGI

1. Bahan

a. Simplisia yang merupakan komposisi dari Jamu Nifas antara lain: *Curcumae rhizoma* (Temulawak), *Litseae odoriferae folium* (Daun Trawas), *Zingiberis rhizoma* (Rimpang Jahe), *Catharmi flos* (Kembang Pulu), *Baeckeae folium* (Jungrahab), diperoleh dari pabrik jamu dan farmasi PT. Sido Muncul Semarang. Semua simplisia kemudian dikirim ke Herbarium Bogoriense, Puslitbang Biologi LIPI Bogor, untuk dideterminasi.

b. Bahan kimia : Metanol p.a, Etil asetat p.a, Kloroform p.a, Amonium hidroksida, Asam asetat, Heksan, 1-Octanol extra pure (semua dari E. MERCK) dan Aqua bidestilata.

2. Alat

Blender, ayakan, timbangan analitik merk Sartorius 2402, shaker, rotavapor merk Buchi R-114, silika gel GF 254 (E.Merck) siap pakai, bejana KLT, saringan Buchner, pipa kapiler, corong pisah, sentrifius, kertas saring, alat-alat gelas lainnya dan oven.

3. Cara kerja

a. Pembuatan ekstrak metanol: simplisia yang telah dideterminasi dihaluskan dengan menggunakan blender. Serbuk masing-masing ditimbang sebanyak 100 gram, dimaserasi dengan cairan penyari metanol p.a sampai cairan penyari bening. Filtrat hasil maserasi dipekatkan dengan rotavapor pada suhu 30°C sampai kental. Kemudian diuapkan hingga kering, diudara terbuka dalam lemari asam dengan penarik udara.

b. Racikan jamu dibuat sendiri dengan menimbang masing-masing serbuk simplisia seperti yang tertera pada etiket jamu nifas, kemudian diekstraksi dengan cara yang sama seperti diatas. Formula Jamu Nifas terdiri dari: Curcumae rhizoma 9%, *Litseae odoriferae* folium 9%, Zingiberis rhizoma 5%, Catharmi flos 9%, dan Baeckeae folium 10%.

c. Pemilihan Sistem Eluasi yang sesuai: ekstrak kering metanol masing-masing simplisia ditimbang seberat 250 mg, dilarutkan dengan metanol p.a sampai 25ml. Kemudian ditotolkan dengan menggunakan pipa kapiler pada lempeng KLT Silika gel GF 254 (E.Merck), kemudian dieluasi. Berbagai sistem eluasi yaitu campuran pelarut organik dalam berbagai perban-dingan dicoba hingga diperoleh pemisahan antara bercak yang cukup baik. Bercak kemudian diamati dibawah sinar UV dengan panjang gelombang 366 nm, kemudian hitung nilai Rf menggunakan persamaan 2.

d. Koefisien Partisi: digunakan sistem oktanol-air yaitu campuran oktanol dan air sama banyak, digojok selama 24 jam. Kemudian kedua cairan dipisahkan dengan corong pisah sehingga diperoleh fase oktanol jenuh air dan fase air jenuh oktanol.
Uji partisi: Ekstrak metanol kering masing-masing sebanyak 500 mg dilarutkan dalam 15 ml fase yang dapat melarutkan lebih baik, kemudian ditambah 15 ml fase kedua, digojok selama 3 jam. Fase air dipisahkan dari oktanol dengan menggunakan corong pisah. Kemudian masing-masing fase disentrifus pada 2000 rpm selama 15 menit. Larutan dalam masing-masing fase dikumpulkan, dikeringkan pada 35°C dalam lemari pengering, ditimbang hingga berat konstan. Ekstrak kering dilarutkan dalam metanol dan dilakukan KLT dengan sistem eluasi terpilih untuk masing-masing ekstrak simplisia tersebut. Koefisien partisi dihitung berdasarkan perbandingan zat dalam fase oktanol dan fase air menggunakan persamaan 1.

HASIL

1. Ekstrak Metanol dari Komponen Jamu Nifas

Curcumae rhizoma (Temulawak), Litseae odoriferae folium (Daun Trawas), Zingiberis rhizoma (Rimpang Jahe), dan Baeckeae folium (Jungrahab), mengandung minyak atsiri, sedangkan Catharmi flos (Kembang Pulu) tidak mengandung minyak atsiri. Lihat Tabel 1.

Tetranthera brawas Bl dari familia Lauraceae. Daunnya dapat digunakan sebagai obat penambah nafsu makan, membuat tenang, kolagogum. Di daerah Bogor daun trawas setelah diremas-remas kemudian dioleskan pada payudara ibu untuk menambah keluarnya air susu [7]. Kandungan kimia daun trawas terdiri dari minyak atsiri kuning muda (minyak trawas yang mengandung *nonyleen*, *Methyl keton* [CH_2=$CH(CH_2)_7$-$COCH_3$], *Methyl-n-nonyleen-Carbinol*) [8].

c. Rimpang Jahe (Zingiberis rhizoma)

Rimpang jahe berbau aromatik dan mempunyai rasa yang pedas [9]. Secara tradisional, jahe yang mentah diparut digunakan sebagai obat oles untuk mengobati pembengkakan atau rematik dan kadang-kadang juga untuk mengobati sakit kepala. Selain itu, juga digunakan sebagai karminatif, peluruh dahak (obat batuk), peluruh haid, peluruh keringat, pencegah mual, penambah nafsu makan dan penurun tekanan darah [5,7]. Rimpang jahe dengan minyak atsiri 2%--3% yang mengandung zingiberen, feladren, kamfer, limonen, borneol, sineol, sitral dan zingiberol. Juga terdapat minyak damar yang mengandung zingeron [9].

d. Kembang Pulu (*Catharmi flos*)

Kembang Pulu merupakan bunga majemuk *Catharmus tinctorius* L, mempunyai bau agak aromatik dan rasa agak pahit, biasanya digunakan sebagai laksatif, astringen. Kembang pulu mengandung zat warna merah cartamin (catharmic acid $C_{12}H_{22}O_{11}$), zat warna kuning (saflower $C_{22}H_{30}O_{15}$), lendir dan minyak lemak [10].

e. Jungrahab herbae (*Baeckeae folium*)

Jungrahab *herbae* merupakan bagian seluruh tumbuhan yang digunakan untuk pengobatan, mempunyai bau khas aromatik dan rasa agak pahit [6]. Digunakan sebagai diuretik, obat sakit perut, obat demam, demam nifas, abortivum, untuk mengusir serangga dengan baunya, bagian jamu (untuk membersihkan setelah haid), gigitan ular, malaria, cacing gelang, susah kencing, dan pengobatan pasca persalinan. Kandungan kimia dari Jungrahab adalah minyak atsiri (eugenol, kariofilen), damar, zat samak, glikosida pahit [6]

Hasil penelitian ini diharapkan dapat memberikan informasi ilmiah mengenai kemampuan suatu kandungan komponen jamu untuk berpartisi melalui membran biologik. Lebih jauh lagi untuk mengetahui koefisien partisi dan karakteristik kandungan komponen jamu nifas yang dapat berpartisi kedalam sistem *octanol* : air.

METODOLOGI

1. Bahan

a. Simplisia yang merupakan komposisi dari Jamu Nifas antara lain: *Curcumae rhizoma* (Temulawak), *Litseae odoriferae folium* (Daun Trawas), *Zingiberis rhizoma* (Rimpang Jahe), *Catharmi flos* (Kembang Pulu), *Baeckeae folium* (Jungrahab), diperoleh dari pabrik jamu dan farmasi PT. Sido Muncul Semarang. Semua simplisia kemudian dikirim ke Herbarium Bogoriense, Puslitbang Biologi LIPI Bogor, untuk dideterminasi.

b. Bahan kimia : Metanol p.a, Etil asetat p.a, Kloroform p.a, Amonium hidroksida, Asam asetat, Heksan, 1-Octanol extra pure (semua dari E. MERCK) dan Aqua bidestilata.

2. Alat

Blender, ayakan, timbangan analitik merk Sartorius 2402, shaker, rotavapor merk Buchi R-114, silika gel GF 254 (E.Merck) siap pakai, bejana KLT, saringan Buchner, pipa kapiler, corong pisah, sentrifius, kertas saring, alat-alat gelas lainnya dan oven.

3. Cara kerja

a. Pembuatan ekstrak metanol: simplisia yang telah dideterminasi dihaluskan dengan menggunakan blender. Serbuk masing-masing ditimbang sebanyak 100 gram, dimaserasi dengan cairan penyari metanol p.a sampai cairan penyari bening. Filtrat hasil maserasi dipekatkan dengan rotavapor pada suhu 30°C sampai kental. Kemudian diuapkan hingga kering, diudara terbuka dalam lemari asam dengan penarik udara.

b. Racikan jamu dibuat sendiri dengan menimbang masing-masing serbuk simplisia seperti yang tertera pada etiket jamu nifas, kemudian diekstraksi dengan cara yang sama seperti diatas. Formula Jamu Nifas terdiri dari: Curcumae rhizoma 9%, *Litseae odoriferae* folium 9%, Zingiberis rhizoma 5%, Catharmi flos 9%, dan Baeckeae folium 10%.

c. Pemilihan Sistem Eluasi yang sesuai: ekstrak kering metanol masing-masing simplisia ditimbang seberat 250 mg, dilarutkan dengan metanol p.a sampai 25ml. Kemudian ditotolkan dengan menggunakan pipa kapiler pada lempeng KLT Silika gel GF 254 (E.Merck), kemudian dieluasi. Berbagai sistem eluasi yaitu campuran pelarut organik dalam berbagai perban-dingan dicoba hingga diperoleh pemisahan antara bercak yang cukup baik. Bercak kemudian diamati dibawah sinar UV dengan panjang gelombang 366 nm, kemudian hitung nilai Rf menggunakan persamaan 2.

d. Koefisien Partisi: digunakan sistem oktanol-air yaitu campuran oktanol dan air sama banyak, digojok selama 24 jam. Kemudian kedua cairan dipisahkan dengan corong pisah sehingga diperoleh fase oktanol jenuh air dan fase air jenuh oktanol.
Uji partisi: Ekstrak metanol kering masing-masing sebanyak 500 mg dilarutkan dalam 15 ml fase yang dapat melarutkan lebih baik, kemudian ditambah 15 ml fase kedua, digojok selama 3 jam. Fase air dipisahkan dari oktanol dengan menggunakan corong pisah. Kemudian masing-masing fase disentrifus pada 2000 rpm selama 15 menit. Larutan dalam masing-masing fase dikumpulkan, dikeringkan pada 35°C dalam lemari pengering, ditimbang hingga berat konstan. Ekstrak kering dilarutkan dalam metanol dan dilakukan KLT dengan sistem eluasi terpilih untuk masing-masing ekstrak simplisia tersebut. Koefisien partisi dihitung berdasarkan perbandingan zat dalam fase oktanol dan fase air menggunakan persamaan 1.

HASIL

1. Ekstrak Metanol dari Komponen Jamu Nifas

Curcumae rhizoma (Temulawak), Litseae odoriferae folium (Daun Trawas), Zingiberis rhizoma (Rimpang Jahe), dan Baeckeae folium (Jungrahab), mengandung minyak atsiri, sedangkan Catharmi flos (Kembang Pulu) tidak mengandung minyak atsiri. Lihat Tabel 1.

inconsistent results, Nutrition Committee of the American Heart Association recently concluded that soy protein decreases serum cholesterol in rabbits but not in human.

It is evidence that either soy protein, its constituent amino acids, a non-protein constituent or a combination of these factors alters cholesterol blood lipid profiles in a variety of species. It is likely that the mechanism varies depending on the species. Available evidence is thus far inconclusive with regard to any one component responsible. It is thus likely that the cholesterol-lowering effect of soy is the result of a combination of components acting together, providing a matrix by which cholesterol metabolism is altered and resulting in depressions in blood cholesterol concentration.

Numerous attempts have been made to explain the mechanisms by which dietary proteins and amino acids alter the concentrations of serum total and LDL cholesterol [6,17]. Sirtori et al, 1993 also observed that similar phenomenon [46].

Numerous studies have been carried out on the pattern of sterol biosynthesis, bile acid formation and lipoprotein secretion in different animal models treated with the soybean diet or with the corresponding diet with casein. In the rat models, some researchers were able to confirm a significant hypocholesterolemic activity of soybean proteins, when given with a high fat, cholesterol-free regiment [47]. The hypocholesterolaemic effect was not associated with any significant alteration in the apolipoprotein pattern. Bile acid formation, as assessed from liver 7 α-hydroxylase activity, was unaffected. A significant increase in the liver acyl coenzyme A-cholesterol acyltransferase (ACAT) activity could be observed.

Most of the published studies on the mechanism of the soybean protein effect have been related to the pattern of sterol absorption and/or excretion. In the rabbits, an increased neutral sterols, not bile acids, excretion after the shift from a moderately low-fat diet with casein, to one with soy protein [48]. Huff and Carroll confirmed these findings, while also describing an increase bile acid excretion in soybean treated rabbits [49].

More complete data on the pattern of cholesterol synthesis, distribution and excretion in swine were provided by Kim et al [50,51]. In early study, the authors were failed to demonstrate any differential activity of proteins on cholesterol absorption and on neutral sterol and bile acid excretion [50]. Despite of the reduction of cholesterolemia, swine did not appear accumulate cholesterol in any body tissue. More recently [51], the same authors could definitely show an increased fecal excretion, mostly of neutral sterols, but also of bile acid, soon after changing the dietary protein from casein to soybean.

HYPOCHOLESTEROLEMIC EFFECTS AND AMINO ACIDS COMPOSITION

It is now well established that the type of dietary protein influences serum cholesterol levels in rabbits and in a number of other animal species, but the underlying mechanism of this phenomenon has not yet been elucidated [52]. Several studies from Carroll and co-workers have indicated that differences in amino acid composition are at least partly responsible for the observed effects [53].

The amino acid composition of vegetable, as compared with animal proteins, shows significant differences. Vegetables proteins are low in sulphur-containing amino acids (e.g. methionine) and comparatively rich in arginine. The arginine/lysine ratio of soybean proteins is markedly higher than that of casein. Lysine reportedly inhibits liver arginase [54], thus possibly allowing an increased production of the arginine-rich protein, the major cholesterol vehicle in rabbits [55]. High lysine diets have been shown to raise plasma cholesterol concentration [56]. Other study as shown in Table 3 found that the lysine/arginine ration does indeed affects atherosclerosis [57].

Table 3. Influence of Experimental Atherosclerosis of Lysine (L) or Arginine (A) Added to Soy Protein or Casein (Average of 3 Studies) #

Group	No	Cholesterol (mg/dl)	Average Atherosclerosis	
			Arch	Thoracic
Casein (C)	20/28	268	1.61	1.07
Soy (S)	25/28	157	0.70	0.40
Casein + A	20/28	254	1.30	0.94
Soy + L	25/28	209	0.94	0.71
C vs. CA		-5%	-17%	-12%
S vs. SL		+33%	+34%	+78%

Source: Krichevsky, D. (2001).

Huff and Carroll gave amino acid mixtures to rabbits, e.g. 50/50 casein-soy protein, added with the essential amino acids from one or from the other diet [49]. Mixtures with different sources of non-essential nitrogen, and/or with different ratios of essential to non-essential amino acids, failed to determine changes in cholesterolaemia or atherosclerosis development. This studies quite clearly indicate that the predominant protein (soy or casein) is the determinant of the final cholesterolaemia.

Susilowati Herman et al. [13] in their studies using rabbits reported that enrichment of 1% methionine to the isolate soy protein diet, isolate green bean protein diet, and isolate mungbeans protein diet, increased serum cholesterol levels of the rabbits by 50-100% compared with the isolate of those plant protein diets without additional of 1% methionine. The isolate mungbean protein diet plus 1% methionine showed the highest increment of serum cholesterol levels. They also reported that diets containing isolate plant proteins were less atherogenic compared with animal proteins. Comparing amongst isolates plant proteins; isolate mungbean protein was the most atherogenic. Comparison between isolate beans proteins and their intact beans, it was clear that the isolate beans protein was more cholesterolemic and atherogenic compared with their beans protein. It was may be due to the fiber and isoflavoned content of isolates was lower compared with the beans. Amongst isolates animal proteins, isolates fish protein was the most atherogenic.

Whatever the case, it is of interest to note that protein hydrolates of casein or soybean protein have the same effect on plasma cholesterol as intact proteins [53]. In contrast, amino acids mixtures, corresponding to the same protein seem to have a different effect on the rabbit's model (Table 4).

Miettinen and Tarpila find the expected changes in sterol excretion in the presence of a high dietary fiber intake, are consistent with a mode of action unrelated to non-absorbable dietary components [58]. Similar findings have also been described by Huijbregts et al. also in vegetarians [59]. A reduced bile acid excretion, with an increased dehydroxylation of cholic acid in the gut, possibly favoring bile acid conservation, was in fact described in these subjects.

Table 4. Effects of Casein, Soy Protein, Tyrosine Hydrolysates and Corresponding Amino-Acid Mixtures on Rabbits Cholesterolaemia [#]

Group	Plasma cholesterol (mg/dl)		
	Initial	14 days of diet	28 days of diet
Casein:			
Intact	40±5	165±29	213±53
Tyrosine hydrolysate	66±15	130±18	178±30
Amino-acid mixtures	54±8	159±39	213±42
Soy protein:			
Intact	61±7	51±8	69±12
Tyrosine hydrolysate	60±9	81±11	41±8
Amino-acid mixtures	56±27	127±20 *	124±30 *

* *Significant different p<0.01, versus intact and tyrosine hydrolysate groups.*
\# *From Huff, 1977.*

Grundy and Abraham failed to note any significant change in the pattern of fecal steroid excretion of type II hyperlipoproteinemic subjects treated with soybean diet regimen [60]. The mechanism of the soybean-protein diet is markedly different from that of drug affecting steroid excretion into the gut lumen, and of drugs interfering with the bile acid reabsorption. The marked plasma cholesterol reduction, occurring in the first 10 days after changing the dietary protein, was not accompanied by any marked alteration in the fecal steroid excretory pattern.

The reported studies on the mechanism of the soy protein induced plasma cholesterol reduction show, of course, considerable variability between the animal and human models. Reduction in plasma cholesterol concentration was, however, observed in both cases only when cholesterolaemia was elevated, either by an experimental diet in animals, or spontaneously in type II patients. In animal models, an increased fecal steroid excretion was constantly found with the soybean protein diet.

The obvious difference between the animal and the human models is that, in the animal models, hypocholesterolemia is induced by feeding an unnatural diet. The failure to note increased sterol excretion both in vegetarians and type II patients treated with soybean diet reminds, in a way, of the observation by Grundy and Ahrens in type II patients treated with polyunsaturated fatty acids diets. Also in this case, no consistent changes in fecal sterol excretion followed the plasma cholesterol reduction [61]. Conclusion the mechanisms of the hypocholesterolaemic activity of textured soy proteins are difficult to draw.

DISCUSSION

Dietary protein type influences experimental atherogenesis in rabbits even in the absence of additional dietary cholesterol. White fish meal protein is much more atherogenic in rabbits than dietary soya or milk powder. Dietary protein type is a potent regulator of serum cholesterol levels and soybean protein exerts a hypocholesterolemic effect compared with casein [62,63,64,65]. There is a large body of research indicating that a total substitution of soy protein lowers blood cholesterol concentrations in humans [6,66]. The substitution of dietary protein for carbohydrate favorably alters the blood cholesterol pattern by significantly reducing plasma total cholesterol and LDL-cholesterol in the presence of a normal fat intake [67]. Replacing texturized soy-protein granules for animal protein in the diets of hypercholesterolemic men resulted in significant depression in total and low-density-lipoprotein (LDL)-cholesterol concentration [33,68].

The main mechanism by which cholesterol is eliminated from the body is by its conversion into bile acids, which are then excreted in the feces. Interruption of the enterohepatic circulation of bile acids leads to up-regulation of cholesterol 7 α-hydroxylase and HMG CoA reductase, which are the rate limiting enzymes for bile acids and cholesterol synthesis. Soybean and other legumes have a propensity to lower LDL cholesterol by this mechanism. Duane demonstrated that the cholesterol-lowering effect of legumes is not just confined to soybeans [69]. The lack of cholesterol in soy protein and relatively high polyunsaturated to saturated fatty acids are also factors in the overall hypocholesterolemic effect of soy protein, as these will contribute to a positive effect on blood lipid. Reducing significantly cholesterol intake will lower LDL cholesterol and it has been shown that soy reduces the absorption of cholesterol and bile acids [70]. Susilowati et al. [13] demonstrated that cholesterol-lowering effect of soybeans is higher compared with other legumes.

For the rabbit model, the mechanism underlying the cholesterol lowering activity of soy protein compared to casein, can be described as follows [71]. Soybean protein decreases the absorption of intestinal cholesterol which is of endogenous and/or exogenous origin and probably also reduces the reabsorption of bile acids [72,73]. Thus soybean protein causes a diminishes feedback inhibition of the hepatic conversion of cholesterol into bile acids. More cholesterol will be channeled into the bile acid synthetic pathway. This, in turn, tends to deplete liver cholesterol pools. Liver cholesterol concentrations have indeed been shown to be lower in rabbits fed soybean protein diet than in their counterparts fed casein [72]. Thus dietary soybean protein reduces liver cholesterol in rabbits. The liver responds by an increase in the number of LDL receptors and by enhancing *de novo* cholesterol synthesis. Indeed Sirtori, Galli, Lovati et al 1984 [74] have shown that the binding of apoprotein B containing β VLDL particle to liver membranes of rats is increased when the donor animals have been fed a cholesterol-rich diet containing soybean protein, compared to casein. Stimulation of hepatic cholesterol synthesis in soybean-protein fed animals has been demonstrated both directly and indirectly. Liver microsomal HMG-CoA reductase activity has been found to be increased in rats fed soybean protein compared to casein [75]. Cholesterol turnover is much faster in rabbits fed soybean protein compared to casein [72].

The increase number of LDL receptors induce by soybean protein is responsible for the fall in serum cholesterol. The LDL cholesterol taken up by the liver can be used for bile acids synthesis. However, in order to prevent the body from depletion of cholesterol, *de novo* synthesis has to be activated. A new steady state will be reached, in which hepatic cholesterol synthesis and faecal excretion of bile acids is also increased. Thus, cholesterol turn over is enhanced. As this new steady state serum cholesterol is low and the number of LDL receptors high. It can be concluded, from the rabbits studies that the cholesterol lowering activity of soybean protein may well reside in its ability to interrupt the entero-hepatic cycle of cholesterol and bile acids. How this effect is brought about is not yet known.

CONCLUSIONS

In general, replacement of saturated by polyunsaturated fatty acids is the single most powerful dietary intervention able to lower serum total cholesterol levels in man. A substitution of animal protein for vegetable protein has relatively small influences on serum cholesterol. However, the nutritional effects on serum cholesterol concentrations should not be considered insignificant. The cholesterol-lowering effect of legumes is not just confined to soybeans, but the highest effect was found in soybean. The mechanisms by which the dietary components affect serum lipoprotein concentration are by no means clear. Nevertheless, it appears obvious that different dietary components act through different pathways. This would imply that cholesterolaemic effects of different nutrients are additive. Indeed, suggestive evidence for this has been obtained experimentally [76]. It is almost certain that the cholesterol-lowering effect of soy protein and other legumes is accounted for by multiple factors. Soy protein appears to up-regulate the expression of LDL receptors [77,78]. It is important to study dietary patterns rather than specific dietary components. The most reasonable advice for healthy people is eat a variety food in moderate amount, and balanced.

REFERENCES

1. Simoons, F.J. (1991). Food in China. A Cultural and Historical Inquiry CRC Press Inc.Boca FL.
2. Young, V.R., Pellet, P.L. (1987). Protein Intake and Requirement with Reference to Diet and Health. Am.J.Clin.Nutr. 45: 1323-1343.
3. Young, V.R., Wayler, A., Garza, C., Steinke, F.H., Muray, E., Rand, W.M. and Schrimsaw, N.S. (1984). A Long Term Metabolic Balance Study in Young Men to Assess the Nutritional Quality of An Isolated Soy Protein and Beef Proteins. Am.J.Clin.Nutr. 39: 8-15.
4. Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K. and Okubo, K. (1991). Malonyl Isoflavone Glycosides in Soybean Seeds (Glycine max Merrill). Agric.Biol.Chem. 55: 2227-2233.
5. Lapre, J.L., West, C.E., Lovati, M.R., Sirtori, C.R., Beynen, A.C. (1988). Dietary Animal Proteins and Cholesterol Metabolism in Rats. Internat.J.Vit.Nutr.Res. 39: 93-100.
6. Carroll, K.K. (1991b). Review of Clinical Studies on Cholesterol-lowering Response to Soy Protein. J.Am.Diet.Assoc. 91: 820-7.
7. Kritchevsky, D. (2001). Diets and Atherosclerosis. The J. of Nutr. Health & Aging. 5 (3):155-159.

8. Kritchevsky, D. and Czarnecki, KS. (1981). Dietary Proteins and Atherosclerosis. Soy Protein in the Prevention of Atherosclerosis. (Eds) G.C.Descovich and S.Lenzi. MTP Press Limited, International Medical Publisher, Lancaster-Boston-The Hague.
9. Clarkson,T.B., Shirvely, C.A., Weingand, K.W. (1988). Animal models of diets-induce atherosclerosis. Comparative Animal Nutrition. Vol.6. A.C. Beynen and C.E. West (eds). Karger, Bases, Munchen, Paris, London, New York, New Delhi, Singapore, Tokyo, Sydney.
10. Kritchevsky, D. (1979). Vegetables protein and atherosclerosis. J.Am.Oil Chem.Soc. 56:135-40.
11. Carrol, K.K. and Hamilton, R.M.G. (1975). Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. J.Food.Sci. 40:18-23.
12. Carroll, K.K. (1982). Hypercholesterolemia and atherosclerosis: effects of dietary protein. Fed.Proc. 41: 2792-6.
13. Susilowati Herman, Endi Ridwan, Herman Sudiman, Hanny Wijaya, and Tonny Ungerer (2001). The influence of protein on hyperlipidemic and atherosclerosis in rabbits. Presented at The 17th International Congress of Nutrition, Vienna, August 27-31, 2001.
14. Khosla, P., Samman, S., and Carroll, K.K. & Huff, M.W. (1989). Turnover of ^{125}I-VLDL and ^{131}I-LDL apolipoprotein Bin rabbits fed diets containing casein or soy protein. Biochem.Biophys.Acta 1002: 157-163.
15. Khosla, P., Samman, S., and Carroll, K.K. (1991). Decrease receptor-mediated LDL catabolism in casein –fed rabbits proceed thre increase in plasma cholesterol levels. J. Nutr. Biochem. 2:203-209.
16. Sugano, M., Ishida, Y. and Koba, K. (1988). Protein-fat Interaction on Serum Cholesterol Level, fatty Acid Desaturation and Eicosanoid Production in Rats. J.Nutr. 118:548-554.
17. Choi-Y-S. Imazato, Y., Ikeda, I. and Sugano, M. (1989). The combined Effects of Dietary Proteins and Fish Oil on Cholesterol Metabolism in Rats of Different Ages. Lipid. 24: 506-510.
18. Eklund, A. and Sjoblon, L. (1989). Effect of Dietary Proteins on Hepatic and Plasma Lipids, and Some Properties of Major Plasma Lipoprotein Fractions During Dietary-induced Hypercholesterolemia in Male rats. Biochem.Biophys.Acta. 877:127-134.
19. Kritchevsky, D., Tepper, S.A., Davidson, L.M., Fisher, E.A. and Kurdfeld, D.M. (1989). Experimental Atherosclerosis in Rabbits Fed Cholesterol free-Diets.13. Interaction of Proteins and fats. Atherosclerosis. 75:123-127.
20. Beynen, A.C. (1990). Mode of Cholesterolemic Action of Dietary Proteins. Monogr. Atheroscler. 16:153-159.
21. Beynen, A.C. (1990). Influence of Dietary Protein on Serum Cholesterol Concentrations and Atherosclerosis. XIV/XV: 55-60.
22. Sugano, M., Yamada, Y., Goto, S. and Yoshida, K. (1990). Aortic Fibro fatty Type Atherosclerosis from Thrombus in Normolipidemic Rabbits. Atheroscler. 18: 43-57.
23. Yamashita, J., Fujita, J., Kamimura., M. and Hayashi, S. (1990). Different Effects of Soy Protein on Cholesterol Metabolism in Rats.Monogr. Atheroscler. 16:36-43.
24. Freyberg, R.H. (1937). Relation of Experimental Atherosclerosis to Diets Rich in Vegetables Protein. Arch.Intern.Med. 59: 660.
25. Meeker, D.R. and Kersten H.H. (1940). Experimental Atherosclerosis and High Protein Diets. Proc.Soc.Exp.Biol.Med. 45: 534.
26. Meeker, D.R. and Kersten H.H. (1941). Effect of High Protein Diets on Experimental Atherosclerosis in rabbits. Arch. Pathol. 31: 147.
27. Hamilton, R.M.G. and Carroll, K.K. (1976). Plasma Cholesterol Levels in Rabbits Fed Low Fat, Low Cholesterol Diets. Effects of Dietary Protein, Carbohydrate, and Fiber From Different Sources. Atherosclerosis. 24: 47.
28. West, C.E. and Beynen, A.C. (1989). Comparison of the Hypocholesterolemia Produced by Dietary Casein and Fish Protein. Eiweiss in Nahrung und Ernahrung des Menschen, R. Wenger, B.M. Brandstetter (HRSG.), Wissenschaftliche Verlagsgesellschaft, Stuttgart.
29. Beynen,A.C. (1990). Model of cholesterolemic action of dietary protein. In: Monograph in Atherosclerosis, Vol.16. Dietary Proteins, Cholesterol Metabolism and Atherosclerosis (Sugano,M. & Beynen,A.C.eds.), Karger, Basel, Switzerland.
30. West, C.E., Beynen, A.C., Scholz, K.E., Terpstra, A.H.M., Schutte, J.B., Deuring, K. and Van Gils, L.G.M. (1984). Treatment of Dietary Casein with Formaldehyde Reduces Its Hypercholesterolemic Effect in Rabbits. J.Nutr. 114, 17.
31. Beynen, A.C., West, C.E., Huisman, J., van Leeuwen, P. and Schutte, J.B. (1985). Differential Cholesterolemic Effects of Dietary Casein and Soy Protein in Pigs: Role of Protein Digestibility. Cholesterol in Health and Disease: Studies in the Netherlands. (Eds). Beynen, A.C., M.J.H. Geelen, M.B.Katan. And J.A. Schouten. Pronsen and Looijen. Wageningen.
32. Porter, S.M., Bakhit, R.M., Essex-Sorlite, D.L., Weingartner, K.R., Chapman, K.M., Nelson, R.A., Prabhudesai, R.M., Savage, W.D., Nelson, A.I., Winter, L.W. and Erdman, J.W. (1993). Depression of Plasma Cholesterol in Man by Consumption of Baked Products Containing Soy Protein. Am.J.Clin.Nutr. 58:501-506.
33. Sirtori, C., Agradi, E., Conti, F., Mantreo, O. and Gatti, E. (1977). Soybean-protein Diet in the Treatment of Type-II Hyperlipoproteinemia. Lancet 1: 275-277.
34. Verrillo, A. de Teresa, A., Giarruso, P.C. and La Rocca, S. (1985). Soybean Protein Diets in the Management of Type-II Hyperlipoproteinemia. Atherosclerosis. 54:321-331.
35. Van Raaij, J.M.A. Katan, M.B. West, C.E. and Hautvast, J.G.A.J. (1982). Influence of diets containing casein, soy isolate, and soy concentrate on serum cholesterol and lipoproteins in middle-aged volunteers. Am.J.Clin.Nutr. 35: 925-34.

36. Van Raaij, J.M.A. Katan, M.B., Hautvast, J.G.A.J. and Hermus, R.J.J. (1981). Effects of casein versus soy protein diets on serum cholesterol and lipoproteins in young healthy volunteers. Am.J.Clin.Nutr. 34: 1261-71.
37. Golberg, A.P., Lim, A., Kolar, J.B., Grundhauser, J.J., Steinke, F.H. and Schonfeld, G. (1982). Soybean protein independently lowers plasma cholesterol levels in primary hypercholesterolemia. Atherosclerosis. 43:355-68.
38. Holmes, W.L., Rubel, G.B. and Hood,S.S. (1980). Comparison of the effect of dietary meat versus dietary soybean protein on plasma lipids of hyperlipidemic individuals. Ahterosclerosis. 36:379-87.
39. Grundy, S.M. and Abrams, J.J. (1983). Comparison of actions of soy protein and casein on metabolism of plasma lipoproteins and cholesterol in humans. Am.J.Clin.Nutr. 38:245-52.
40. Chao,J.S., Yamin, T.T., and Albert, A.W. (1982). Effect of cholestyramine on low density lipoprotein binding sites on liver membranes from rabbits with endogenous hypercholesterolemia induced by wheat starch casein diet. J.Biol.Chem. 257: 3623-3627.
41. Carroll, K.K. (1991a). Vegetables Protein: Potential Lipid-lowering Effects. Medicine North America 4th Series. 17: 2279-2282.
42. Bakhit, R.M., Klein, B.P., Essex-Sorlie, D., Ham, J.O., Erdman, J.W., Jr. and Potter, S.M. (1994). Intake of 25 g of Soybean Protein With or Without Soybean Fiber Alters Plasma Lipid in Man With Elevated Cholesterol Concentration. J. Nutr. 124:213-222.
43. Anderson, JAW. Johnston, B.M. and Newell, M.E.C. (1995). Meta-analysis of the Effects of Soy Protein Intake on Serum Lipids. N.Engl.J.Med. 333:276-82.
44. Caroll, K.K. (1991). Review of clinical studies on cholesterol-lowering response to soy protein. J.Am.Diet. Assoc. 91:820-827.
45. Caroll, K.K., Kurowska, E.M. (1995). Soy consumption and cholesterol reduction: Review of animal and human studies. J.Nutr. 125 (Suppl):S594-S597.
46. Sirtori, C.R, Even, R., and Lovati, M.R., (1993). Soybean protein diet and plasma cholesterol from therapy to molecular mechanisms. Ann.NY.Acad.Sci. 676: 188-201.
47. Pathirana, C., Gibney, M.J. and Taylor, T.G. (1980). Effects of Soy Protein and Saponin on Serum and Liver Cholesterol in Rats. Atherosclerosis. 36:595.
48. Fumagalli, R., Paoletti, R. and Howard, A.N. (1978). Hypocnolesterolemic Effects of Soya. Life Sci. 22, 947.
49. Huff, M.W. and Carroll, K.K. (1980). Effect of the Dietary Protein on Turnover, Oxidation and Absorption of Cholesterol, and on Steroid Oxidation in Rabbits. J.Lipid.Res. 21: 546.
50. Kim,D.N., Lee, K.T., Reiner, J.M. & Thomas, W.A. (1978). Effects of soy protein product on serum and tissue cholesterol concentrations in swine fed high-fed, high-cholesterol diets. Exp.Mol.Pathol. 29: 385-399.
51. Kim,D.N., Lee, K.T., Reiner, J.M. & Thomas, W.A. (1980) Increased steroid excretion in swine fed high-fed, high-cholesterol diets with soy protein. Exp.Mol.Pathol. 33: 25-35.
52. West, C.E., Beynen, A.C., Terpstra, A.H.M., Scholz, K.E., Carroll, K.K. and Woodward, C.J.H. (1983). The nature of Dietary Protein and Serum Cholesterol. Atherosclerosis. 46: 253.
53. Huff, M.W., Hamilton, R.M.G. and Carroll, K.K. (1977). Plasma Cholesterol Levels in rabbits Fed Low Fat, Cholesterol-free, Semi purified Diets: Effects of Dietary Proteins, Protein Hydrolysates and Amino Acid Mixtures. Atherosclerosis. 28: 187.
54. Clattadini, D., Pietropaolo, C., De Cristoforo, D. and D'Ayejello-Caracciolo, M. (1964). In vivo effect of L-lysine on rat liver arginase. Nature (Lond.). 203:643.
55. Rodriguez, J.L., Ghiselli, G.C., Torreggiane, D. and Sirtori, C.R. (1977). Very low density lipoprotein in normal and cholesterol-fed rabbits. 1. Chemical composition of very low density lipoproteins in rabbits. Atherosclerosis. 23 :73.
56. Weisgesberb, B.I., Stary, H.C. and McMillan, G.C. (1964). Effect of lysine deficiency on cholesterol atherosclerosis in rabbits. Exp.Mol.Pathol. 3: 444.
57. Kritchevsky, D., Tepper, S.A., Czarnecki, S.K.,Kurfield, D.M., Strory, D.A. (1983). Effects of animal and vegetables protein in experimental atherosclerosis. In: Gibney M.Y., Kritchevsky, D. (ed). Animal and Vegetable Proteins in Lipid Metabolism and Atherosclerosis. New York, Alan R., Liss Inc.
58. Miettinen, T.A. and tapila, S. (1977). Effect of pectin on serum cholesterol, fecal bile acids and billiary lipids in normolipidemic and hyperlipidemic individuals. Clin.Chim. Acta. 79: 471.
59. Huijbregts, A.W.M., Van Schaik, A., Van berge-Henegouven, G.P. and Van der Werf, S.D.J. (1980). Serum lipids, biliary lipid composition and bile acid metabolism in vegetarians as compared to control subjects. Eur.J.Clin.Invest. 10: 443.
60. Grundy, S.M., and Abrams, J.J. (1983). Comparison actions of soy protein and casein on metabolism of lipoprotein s and cholesterol in humans. Am.J.Clin.Nutr. 38: 245-52.
61. Grundy, S.M., and Abrams, J.J. (1981). Comparison of soy protein and casein on plasma lipids and lipoproteins in man. Presented at the Conference on Soy Protein and Polysacharide, Keynote, Co., USA.
62. Carroll, K.K. and Hamelton, R.M.G. (1975). Effects of Dietary Protein and Carbohydrate on Plasma Cholesterol levels in Relation to Atherosclerosis. J.Food.Sci.40: 18.
63. Nagata, Y., Imaizumi, K. and Sugano, M. (1980). Effects of Soybean Protein and Casein on Serum Cholesterol Levels in Rats. Br.J.Nutr. 44: 113-121.
64. Huang, Y.S., Manku, M.S. and Horrobin, D.F. (1988). The Effects of Dietary Cholesterol on Blood and Liver Polyunsaturated Fatty Acids and on Plasma Cholesterol in Rats Fed various Types of Fatty Acid Diets. Lipids. 19: 664-672.

65. Beyen, A.C. (1990). Mode of cholesterol action of dietary proteins. Monogrr. Atheroscler. 16:158-159.
66. Erdman, J., Fordyce, E. (1989). Soy Products and the Human Diet. Am.J.Clin.Nutr. 49: 725-37.
67. Wolfe, B.M., Piche, L. (1994). Exchanging Dietary Protein for Carbohydrate in Normolipidemic Human Subjects Lowers LDL-cholesterol. Depts.of Medicine and Home Economics (Brescia college), University of Western Ontario, London, Ontario, Canada. In. Posters Abstract. Atherosclerosis X. Montreal, October 1994.
68. Sirtori, C., Gatti, E., Mantero, O. (1979). Clinical Experience With the Soybean Protein in the Treatment of Hypercholesterolemia. Am.J.Clin. Nutr. 32:1645-8.
69. Duane, W.C. (1997). Effects of legume composition on serum cholesterol, biliary lipids, and sterol metabolism in humans. J.Lipid.Res. 38: 1120-1128.
70. Duane, W.C. (1999). Effects of soybean protein and very low dietary cholesterol on serum lipids, biliary lipids, and fecal sterols in humans. Metabolism. 48: 489-494.
71. Beynen, A.C., van der Meer, R., West, C.E., Sugano, M. and Kritchevsky, D. (1986). Possibble mechanisms underlying the differential cholesterolemic effects of dietary casein and soy protein. In A.C. Beymen (ed), Nutritional Effects on Cholesterol Metabolism, Transmondial, Voorthhuizen, 29-45.
72. Huff, M.W.& Carroll, K.K. (1980). Effects of dietary protein on turnover, oxidation, and absorption of cholesterol and on steroid excretion in rabbits. J.Lipid Res. 13:873-884.
73. Kuyvenhoven, M.W., West, C.E., van der Meer, R. and Beynen, A.C. (1986). Fecal steroid excretion in relation to the development of casein-induced hypercholesterolemia in rabbits. J.Nutr. 116:1395-404.
74. Sirtori, C.R., Galli, G., Lovati,M.R., Carrara, P., Bosisio, E. and Galli Kienle, M. (1984). Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. J.Nutr. 114: 1493-1500.
75. Nagata, Y., Ishiwaki, N. and Sugano, M. (1982). Studies on the mechanism of antihypercholesterolemic action of soy protein and soy protein-type amino acid mixtures in relation to the casein counterparts in rats. J.Nutr. 112: 1614-25.
76. Lewis, B., Hammet, F., Katan, M., Kay, R.M., Merkx, I., Nobels, A., Miller, N.E. and Swan, A.V. (1981). Towards an improved lipid-lowering diet: additive effects of changes in nutrient intake. Lancet. 2: 1310-3.
77. Potter, S.M. (1995). Overview of proposal mechanisms for the hypocholesterolemic effect of soy. J.Nutr. 125(Suppl): S606-S611.
78. Baum, J.A., Teng, H., Erdman, J.W.Jr., Weigel, R.M., Klein, B.P., Persky, V.W., Freels, S., Surya, P., Bakhit, R.M., Ramos, M.E., Shay, N.F., Potter, S.M. (1998). Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. Am.J.Clin.Nutr. 68: 545-551.